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Changes in Epidermal Forward Scattering Absorption after UVA or UVA-UVB Irradiation

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Groups of Skh-1 (albino) and Skh-2 (pigmented) hairless mice were irradiated for 125 hr using a modified GE F8T5-BL black light with and without a 3-mm plate glass filter to remove light below 320 nm. The epidermis was examined by forward scattering and by histological section postirradiation at 48 hr, 96 hr, 9 days, and 23 days. Changes in the epidermis of all animals were compared to control groups. Although no differences were seen between Skh-1 and Skh-2 mice, both the magnitude and shape of the forward scattering absorption curves were changed by the irradiation used. In both strains, differences which were detected at 48 hr postirradiation had returned to normal visually by 23 days, with no augmented pigmentation occurring in Skh-2 animals. At 23 days postirradiation, however, residual optical alterations were observed. This phenomenon, detected optically, may be skin acclimatization.

Many studies on the changes induced in skin by irradiation with either long-wave UVA (320–400 nm) or with UVA-UVB combinations have been reported, but they have not dealt with the relationship between the changes observed and the possible effect of this irradiation on further exposures [1–12]. While histological characterization of dermal and epidermal damage, repair processes, edema, and sunburn cells has been extensive, investigations into the underlying optical properties have been neglected.

Greater tolerance to ultraviolet exposure resulting from thickening of the stratum corneum in addition to increased pigmentation has been described by Blum [7,8]. A large decrease in the depth of ultraviolet penetration can be ascribed to even a small increase in the thickness of the stratum corneum [9]. For some time it was felt that epidermal damage to skin was caused primarily by UVB wavelengths (290–320 nm), whereas UVA was relatively harmless [10,11]. After UVA penetration to the dermis was quantiated by Everett et al [12] accounts of specific dermal damage and vascular injury were reported [10,11,13]. UVA-UVB photoaddition or photoaugmentation was proposed [2,3] and the histological effects for each portion of the ultraviolet spectrum were described [1,4,10].

The phenomenon of acclimatization of the skin to UV radiation has been a subject of speculation for some time [9,14]. The concept that skin thickness as a result of UV exposure could be a self-regulated protective mechanism has been proposed [14]. Together with the photoprotection provided by melanin, skin thickening could be a basic mechanism for modification or reduction of further UV damage. The present study details the optical characteristics of acclimatization to ultraviolet radiation in addition to describing the optical changes due to UVA alone. Recently we assessed the protection provided by melanin in human skin by this technique [15]. Using forward scattering spectrophotometry, it is possible to measure and distinguish changes induced by these different wavelengths and to correlate changes seen optically with alterations observed histologically.

MATERIALS AND METHODS

Skh-1 and Skh-2 hairless mice, 5–6 weeks old, were purchased from the Skin and Cancer Hospital, Philadelphia. Skh: hairless-1 (Skh-1) is an albino. Skh: hairless-2 (Skh-2) has dark eyes, lightly pigmented ears, and is capable of modest tanning after UV irradiation. Each group of 6–8 mice was irradiated for 125 hr using a bank of 3 Raymaster black light bulbs (GE F8T5BL modified by Carl Gates Co.) as shown in Fig 1, with and without a 3-mm plate glass filter. Spectral distribution of the lamp was measured using an Optronics Laboratories spectroradiometer. Data was recorded by a Cary Model 14 spectrophotometer (Varian Associates, Palo Alto, Ca). Using the 3-mm plate glass filter, less than 0.117% of the output fell below 320 nm. Unfiltered, 1.43% of the light fell below 320 nm. (Total exposure levels measured with a thermopile and microvoltmeter were 148 joules/cm^2 unfiltered and 113 joules/cm^2 filtered.) Each group of mice was examined postirradiation by gross observation, by forward scattering and histologically at 48 hr, 72 hr, 96 hr, 9 days, and 23 days, along with a group of age-matched unirradiated controls.

At each time period postirradiation, forward scattering absorbance was measured using the diffuse reflectance sphere optics of a Beckman Acta MVI spectrophotometer. The midback irradiated skin of each mouse was removed and the epidermis separated from the dermis [16,17]. Briefly, the mice were sacrificed by cervical dislocation and the skin removed from the dorsal area of the body. After immersion in

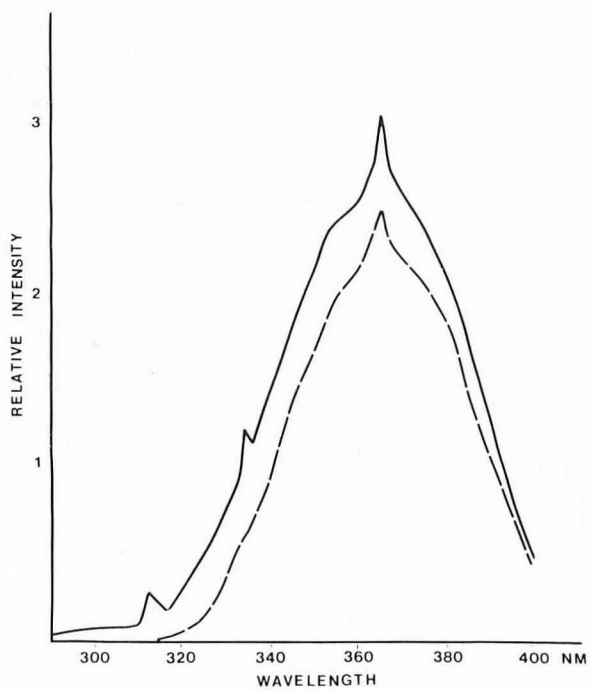

FIG 1. The spectra of the Carl Gates Raymaster black light bulb, with (- - - - -) and without (——) a 3-mm plate glass filter are shown above.

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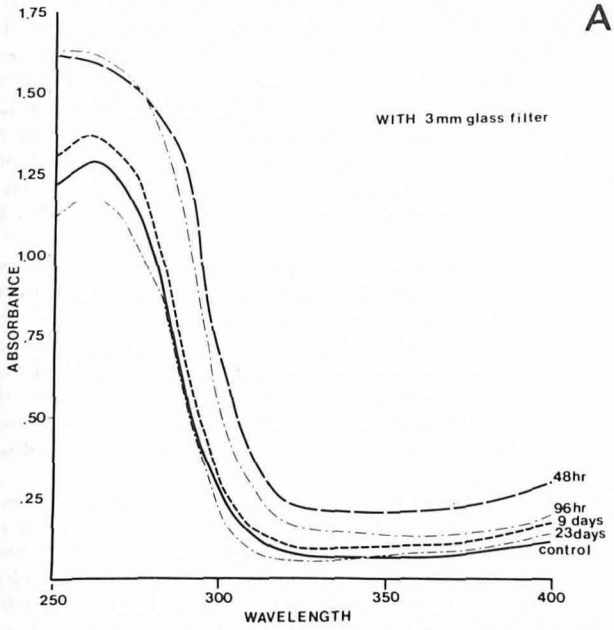
60°C water for 30s, the epidermis was removed intact from the dermal layer by careful blunt dissection. The epidermal layer was then floated onto a UG-5 filter and excess moisture carefully removed. Each piece of epidermis used ranged from 5 cm^2 to 10 cm^2. Forward scattering spectra were obtained with an additional UG-5 filter in the reference beam. This technique, which measures all of the light absorbed, transmitted, and scattered by each skin sample has been described in detail elsewhere [12]. Spectra for each group of 6–8 mice were averaged.

A small section of whole skin from each midback was removed for histological examination, along with a section from a more ventral location. The skin specimens were fixed in buffered 10% formalin, sectioned, and stained with hematoxylin and eosin (H&E). Selected sections were also stained using the Fontana-Masson technique for



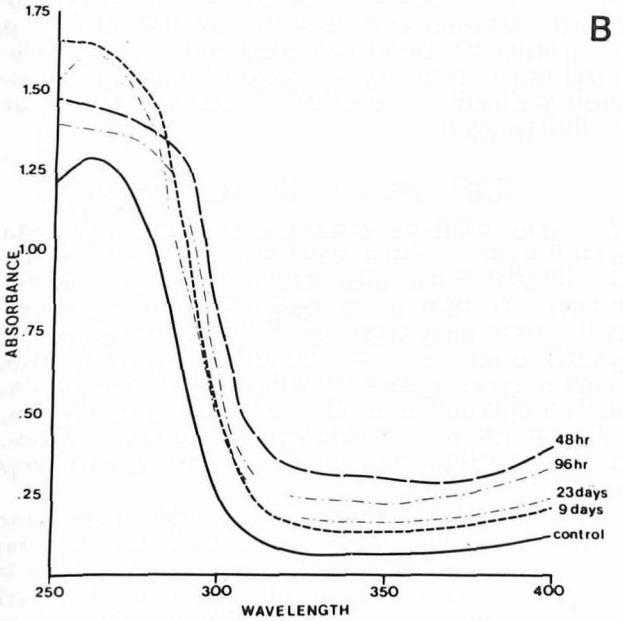


FIG 2. *A*, The forward scattering absorption curves at 48 hr, 96 hr, 9 days, and 23 days postirradiation for the filtered exposure regimen. Increased absorbance seen at 48 hr has returned to control levels by 23 days. *B*, The forward scattering absorption curves at 48 hr, 96 hr, 9 days, and 23 days postirradiation for the unfiltered exposure. Note the change in shape at 48 hr and residual increased absorbance in the 280 nm area at 23 days.

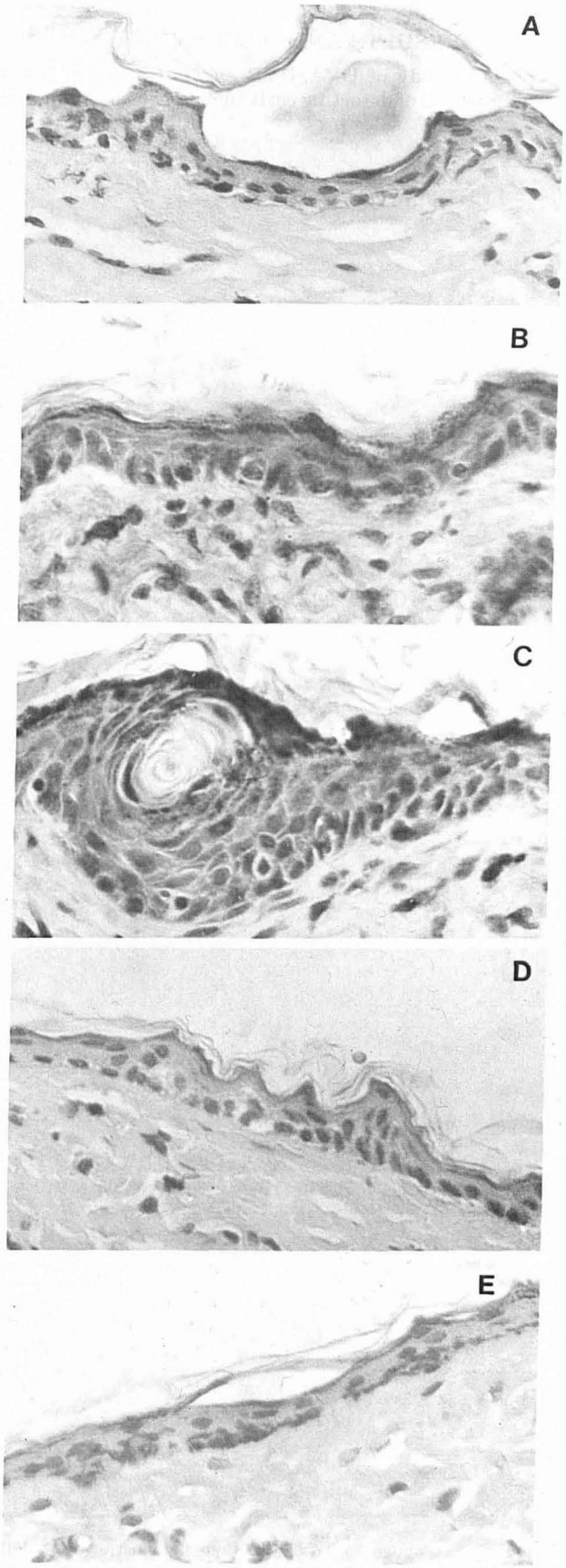


FIG 3. Shown above are typical sections demonstrating the changing epidermal layers. *A*, Control skin; *B*, 48 hr postirradiation, UVA; *C*, 96 hr postirradiation, UVA, note thickened epidermis; *D*, 9 days postirradiation, UVA; and *E*, 23 days postirradiation, UVA.

detection of melanin. Representative areas of these slides were photographed through a Zeiss light microscope.

RESULTS AND DISCUSSION

The present study of UVA/UVB effects was initially conceived as a method of detecting early melanogenesis in the Skh-2 hairless mice. Groups of Skh-1 (albino) mice were included as irradiated controls. However, no augmented pigmentation was observed in the pigmented animals during or after the irradiation regimen given. Early results monitored by forward scattering indicated that dramatic changes were occurring in the epidermis that these changes were detectable in both radiation groups, and were quite different in the UVA-UVB group than in the UVA alone group. Surprisingly, all changes recorded spectrophotometrically for the Skh-2 (pigmented) groups were indistinguishable from those of the Skh-1 (albino) groups, and this was borne out by the histological sections stained for melanin.

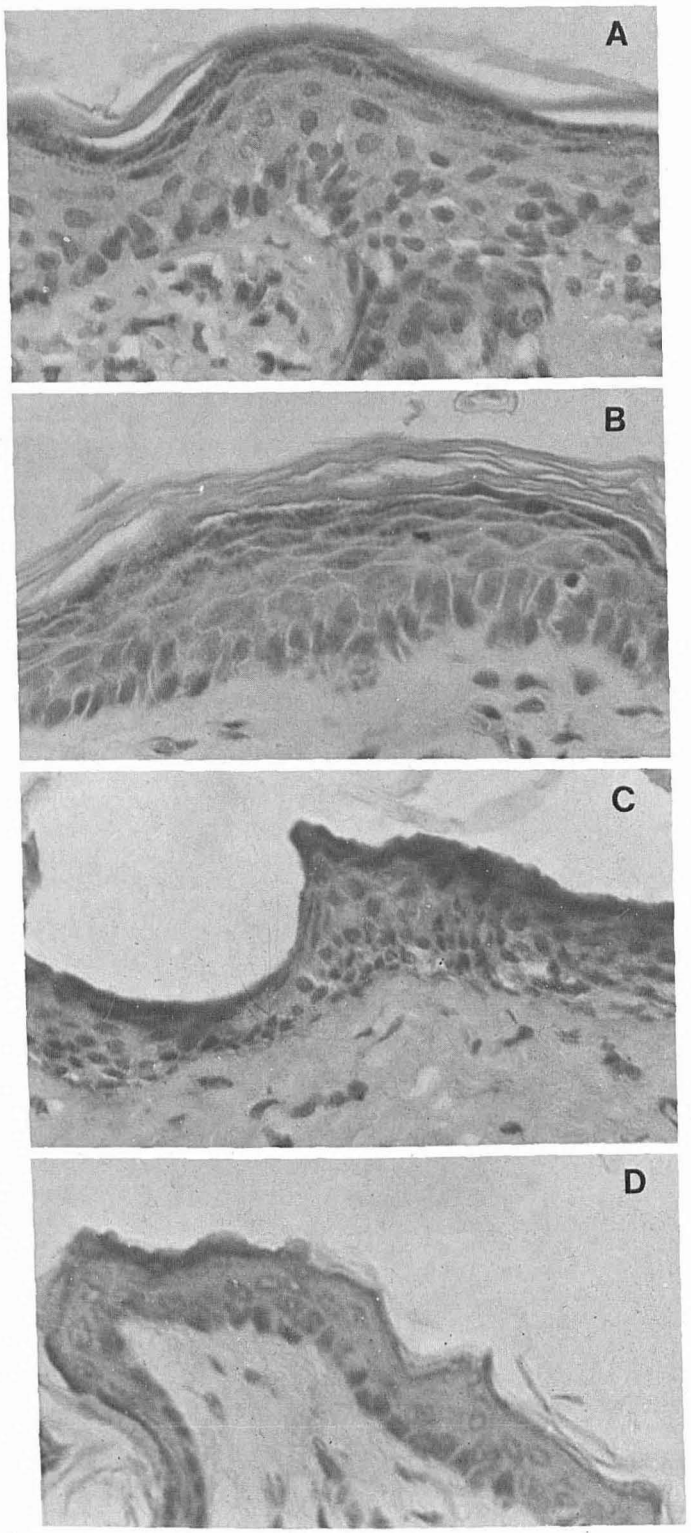


FIG 4. Shown above are typical sections demonstrating the effects of combined UVA-UVB irradiation on hairless mouse epidermis (×560). *A*, 48 hr postirradiation, note formation of granular layer; *B*, 96 hr postirradiation, note: thickened stratum corneum; *C*, 9 days postirradiation; and *D*, 23 days postirradiation.

Shown in Fig 2*A* and 2*B* are the forward scattering analyses of the 125 hr single-exposure mice compared to the absorbance spectrum of the averaged controls. Because there was no difference in the Skh-1 and Skh-2 group results, these data have been combined and averaged. Each line, therefore, is an average spectrum representing data obtained from groups of 12 to 16 mice. Fig 2*A* contains the data resulting from irradiation using the 3-mm plate glass filter: UVA alone. Fig 2*B* shows the data resulting from use of the unfiltered lamp, in which a small component of UVB is present (see Fig 1). In all cases the standard deviation between mice in any group was smaller than 30%.

In Fig 2*A*, at 48 hr postirradiation, there is increased absorbance in all portions of the spectrum. The curve maintains a normal, rounded appearance when compared to the averaged control, at 280 nm. At 96 hr postirradiation, the absorbance in the 300–400 nm region has decreased toward normal, although absorbance in the 280 nm region is still high. At 9 days, there is a drop back to normal in the 280 nm area. At 23 days, the epidermis has a very normal appearance spectrophotometrically.

In Fig 2*B*, with the addition of only a small component of UVB radiation, the results are quite different from those illustrated in Fig 2*A*. At 48 hr postirradiation, there is increased absorption over the entire spectrum, with a noticeable change in the shape of the curve between 250 and 280 nm. At 96 hr postirradiation, although the 300–400 region absorption is decreasing, the shape of the curve is still altered. At 9 days postirradiation, however, the absorbance in the 250–280 nm region increased, becoming more normal in shape. At 23 days, there is not much change from 9 days. Although the epidermis looked normal to the eye, the forward scattering revealed a persistent alteration, a residual increase in absorption in the 250–280 nm region.

HISTOLOGICAL EXAMINATIONS

The skin sections of control animals exhibit a thin stratum corneum with a 2–3 cell thick layer of epidermis. Using the filtered bulb (UVA alone), at 48 hr postirradiation the stratum corneum and epidermis are thicker than the controls, with a small amount of edema present. At 96 hr postirradiation, most of the observed changes are gone. At 9 days postirradiation, the epidermis appears nearly normal histologically (a small amount of edema may still be visible). At 23 days postirradiation, the epidermal sections are indistinguishable from controls, although the collagenous areas of the dermis indicate possible scarring.

Using the unfiltered bulb, the histological alterations can be described as follows. At 48 hr postirradiation, the stratum corneum is thickened, with a thick and distinct granular layer forming. In the dermis, hemorrhaging is evident, with erythrocytes, edema, and acanthotic cells visible. At 96 hr postirradiation, edema, is still evident, with areas of dermal-epidermal separation present. At 9 days postirradiation, the epidermis has become quite thick, with many mitoses present.

At 23 days postirradiation, the epidermis is thinning back to a 2–3 cell thick layer, and edema and capillary dilation are no longer observed. Scarring is detectable in the collagenous areas

of these sections, similar in appearance to the UVA-alone exposure described above. Shown in Fig 3 and Fig 4 are representative sections illustrating the histological alterations observed.

Thus, although the skin sections at 23 days postirradiation (unfiltered) appear to be normal, it is evident from the forward scattering data that some changes remain. The altered shape of the spectral curves at 48 and 96 hr postirradiation could reflect epidermal thickening due to cell proliferation, repair, new DNA and protein synthesis, plus the invasion of circulating cells and fluid. These dynamic activities could also explain the increase in 250–280 nm region absorption at 9 days postirradiation. Woodcock and Magnus [18] have detailed the difficulties of distinguishing between protein and DNA absorption due to their close absorbance maxima. However, by 23 days after irradiation when the histology appears to be returning to normal, the explanation for the persisting increased absorbance above the controls is more speculative. In comparison to the 23 days postirradiation data using the filtered bulbs, the difference is certainly prominent. Since the normal time expected for a cell has been reported as only 4 days [19], one would expect that the radiation damaged cells had already passed into and through the stratum corneum by 23 days.

These results support and extend previous observations that the UVB wavelengths predominantly effect the epidermis, while UVA wavelengths have more dermal effects [1,4]. Nevertheless, we have shown here that the UVA wavelengths also have epidermal consequences which can be measured spectrophotometrically, although they are not as long lasting as those resulting from inclusion of UVB exposure. The damage and repair process for UVA alone and for the UVA-UVB combination seem quite different when the 48 hr and 23 day postirradiation spectra are compared in Fig 2*A* and 2*B*.

We conclude from this study that UVA irradiation has a greater effect on the epidermis than has been reported previously. The addition of a small component of UVB results in quite different changes which can be detected optically. The persisting shape change and increased absorbance seen at 23 days postirradiation when UVB is included may result in what has come to be called skin acclimatization, which will effect the amount of light of all wavelengths that will be able to reach underlying tissues, modifying the effects of subsequent exposures. This has been described clinically in cases of vitiligo by Everett [14], in which thickened keratin layers provide extra protection for depigmented sites. Reactive hyperkeratosis to sunlight could be a normal response regardless of melanin protection.

While the forward scattering technique has resulted in a new method of examining the responses of skin to UV irradiation, it is clear that further dose-response analyses of these phenomena should be pursued along with the dynamics of turnover following initial stimulation. Quantitation and timing of these responses are under examination currently in our laboratories.

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